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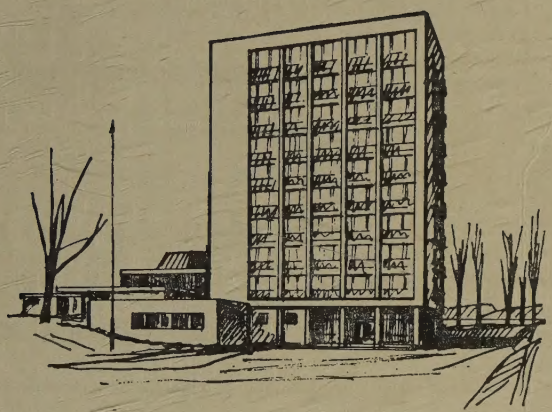

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HEMOGLOBINS AND SERUM PROTEINS IN FOUR NORTH ATLANTIC SEALS, STUDIED BY ELECTROPHORESIS

By

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INTRODUCTION

An investigation of polymorphic characteristics to be applied in population studies of harp seals, *Pagophilus groenlandicus* (Erxleben), and hooded seals, *Cystophora cristata* (Erxleben), was started in 1962. Erythrocyte antigens were tried, but as the seals are caught in the pack-ice of northern waters, it proved difficult to get enough fresh material for these investigations. Although several freezing media were tried, the blood cells ruptured from freezing and thawing, and this complicated the analyses. Attention therefore was shifted to electrophoretic studies of hemoglobins and serum proteins.

The purpose of the present paper is to give a description of hemoglobins and serum proteins of harp seals, hooded seals, ringed seals, *Pusa hispida* (Schreber), and bearded seals, *Erignathus barbatus* (Erxleben), as these proteins appear after starch-/agar-gel electrophoresis. The first step towards identification of populations is to reveal individual differences within species. Therefore most attention has been paid to this problem. The results presented here will form the basis for further investigations.

MATERIAL AND METHODS

An account of the collected material is given in Table 1.

Table 1. Hemoglobins and sera from four North Atlantic seals collected in the years 1963—1964.

	Year	Harp seal		Ringed seal		Bearded seal		Hooded seal	
		Hemo-globins	Sera	Hemo-globins	Sera	Hemo-globins	Sera	Hemo-globins	Sera
Newfoundland area	1963	8	—	—	—	—	—	5	—
» »	1964	200	208	—	—	—	—	155	201
Jan Mayen area . .	1963	166	132	2	1	—	—	166	130
White Sea	1963	105	105	—	—	—	—	—	—
Denmark Strait . .	1964	—	—	—	—	—	—	83	83
Barents Sea	1963	130	103	15	14	46	40	3	2
» »	1964	—	16	—	—	—	—	—	—
Total		69	554	17	15	46	40	412	416

Part of the material of harp and hooded seals has been taken from pups. Blood from mothers and their pups has been sampled to get direct observations on heridity. Except this, all specimens have been sampled at random.

Sampling has been carried out as described for harp seals (NÆVDAL 1966).

Most of the specimens were collected by observers from the Institute of Marine Research on Norwegian commercial sealing vessels in the years 1963—64. The sample from the Denmark Strait was collected on the Danish sealing vessel «Ejnar Mikkelsen», and the sample from White Sea was collected by Soviet scientists.

Because of unfavourable weather and primitive equipment it has proved difficult completely to avoid lysis of erythrocytes and obtain sera free from hemoglobins. Much attention therefore has been given to the problem of analysis of sera containing some hemoglobins.

The electrophoretic technique described by SICK (1961) and MØLLER (1966) with starch-/agar-gel medium and at pH 9.0 was adopted for analysis of all specimens. The electrophoresis was run for 75 minutes to discover also the weaker components on the electrophoretograms. Electrophoresis in «difcoagar» at pH 6.3, as described for the harp seal transferrins (NÆVDAL 1966) has to some extent been carried out for serum proteins.

Proteins were stained with Amido Black 10B (Merck), and the benzidine staining process was used to stain hemoglobin/haptoglobin complexes.

An autoradiographic tecnique (GIBLETT, HICKMAN, AND SMITHIES 1959) used to identify harp seal transferrins (NÆVDAL 1966), has also been applied to hooded seal sera.

RESULTS

HEMOGLOBINS

The electrophoretogram of the harp seal hemoglobins is described elsewhere (NÆVDAL 1966). All specimens from the three other species have given exactly the same pattern as the normal hemoglobins of the harp seal: a major fraction (hem A) moving towards the anode, and a minor fraction (hem A_2) moving towards the cathode at pH 9.0.

Three specimens with deviating hemoglobins, supposed to be genetically controlled, were found among the harp seals. In the other species no deviating specimens could be detected, but here it should be stressed that the number of specimens is much higher for the harp seal than for any of the other species.

It has been possible to compare the electrophoretograms from fresh and frozen hemoglobins of hooded seals because hemoglobin specimens have been available from three living pups kept at the Institute, but no differences were found. Neither were any difference found between hemoglobins from pups and hemoglobins from older animals.

SERUM PROTEINS

General

In the combined starch- and agar-gel medium at pH 9.0, all serum proteins except the γ-globulins moved towards the anode. The γ-globulins were found as a more or less strong diffuse zone on the cathodic side of the point of application. Between the point of application and the albumins, which had the highest electophoretic mobility, the other proteins were found as more or less outstanding bands on the electrophoretogram. Among these fractions, representing the α-and β-globulins, each species presented its own characteristic pattern, and the number of fractions and their distance from the point of application varied between species. Variations were also found within species, especially the precence or absence of fractions, but also in relative strength of fractions. In particular these variations were very complicated for the hooded seal.

Harp seal

A generalized pattern from routine electrophoresis in starch-/agar-gel at pH 9.0, never found in any single animal, is shown in Fig. 1 a. In addition to the γ-globulins and albumins a total of eleven fractions of different strength were seen. Fig. 1 b, c, and d show outlines of the electrophoretograms of three specimens differing in some of the proteins.

The components labelled as I, II, and III represent the proteins demonstrated to be transferrins (Tf A, Tf B, and Tf C respectively). One or two of these fractions were found in each animal, permitting the establishment of six phenotypes (NÆVDAL 1966). Three of these are shown in Fig. 1 b, c, and d (AA, AC, and BB).

Component IV usually is very faint, and often not seen at all (Fig. 1 b and c). This fraction may cause some difficulties for the determination of transferrin groups because it is located close to the Tf C band. However, the IV-band is much weaker than any of the transferrins, so the problem is not serious.

The protein labelled V occurs in all specimens. It is a relatively strong fraction usually giving a well defined band in the gel.

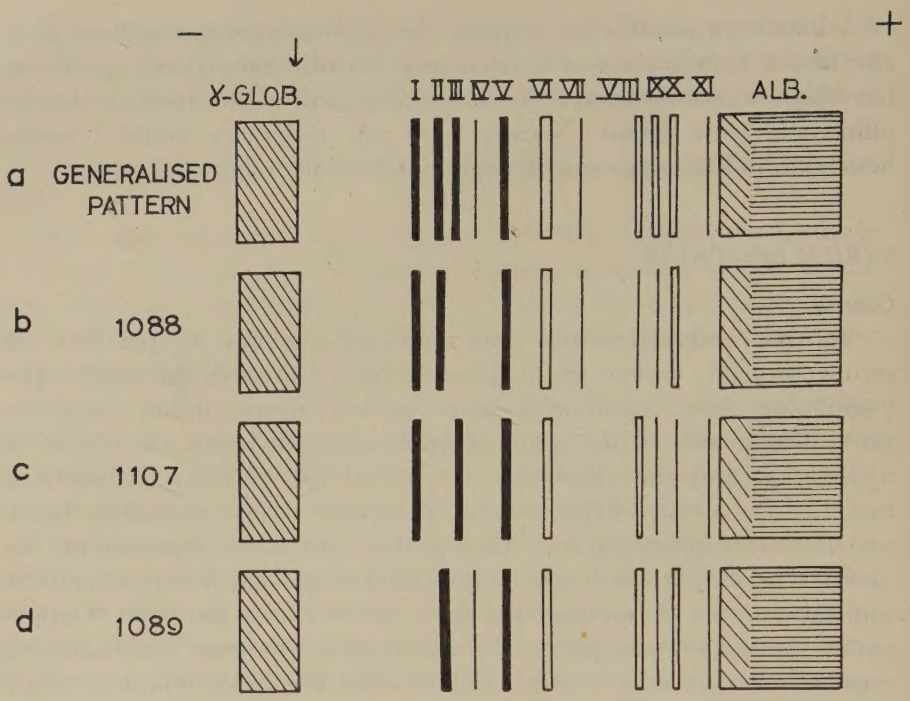

Fig. 1. Outlines of harp seal serum protein patterns by starch-/agar-gel electrophoresis at pH 9.0. Solid bars: sharp and strong bands. Open bars: sharp, but weak bands. Single lines: faint bands. Horizontal shading: strong diffuse zones. Cross hatched: weak diffuse zones. Arrow indicates point of application.

The VI-band represents the haptoglobins. When excess hemoglobins are added to the sera before electrophoresis, this band stains with benzidin. No clear differences were found staining hemoglobinfree sera with Amido Black before and after addition of hemoglobins, but the VI-band was somewhat stronger in the last case. Some times a slightly higher electrophoretic mobility was indicated for the hemoglobin/haptoglobin complexes than for the free haptoglobins. But in contrast to the results for hooded seals (p. 46), this difference was very small.

The VII-band was not seen in all the slides (Fig. 1 c and d), and it usually was very weak. This may represent a case of real difference between specimens, but the absence could also be caused by low protein concentration in the sera.

The three proteins VIII, IX, and X seemed to be closely related. The relative strength of these bands vary to a great extent between individuals, but they are always seen at least as faint bands, and it is very difficult to separate the specimens into groups according to relative strength of these proteins.

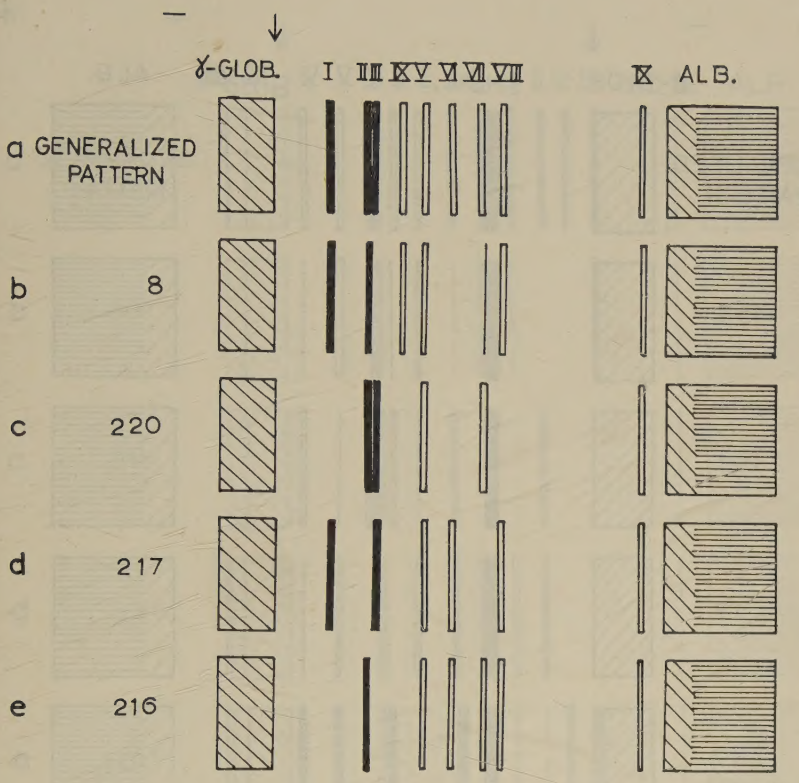

Fig. 2. Outlines of ringed seal serum protein patterns by starch-/agar-gel electrophoresis at pH 9.0. Legend : Fig. 1.

Band XI was seen as a very weak but well defined band on some of the slides. It is located near the albumins, and may be screened by these stronger zones. Therefore it cannot be decided whether the presence or absence of this component represent real difference between individuals.

Ringed seal

A generalized pattern of the starch-/agar-gel electrophoretograms of the ringed seal is shown in Fig. 2 a. The serum of one single animal never showed all fractions. Four individual patterns are given in Fig. 2 b, c, d, and e.

The fractions I, II, and III are very similar to the transferrins of the harp seal, and one or two of these fractions were found in each individual. The same fractions were found in «difcoagar» at pH 6.3, but it was

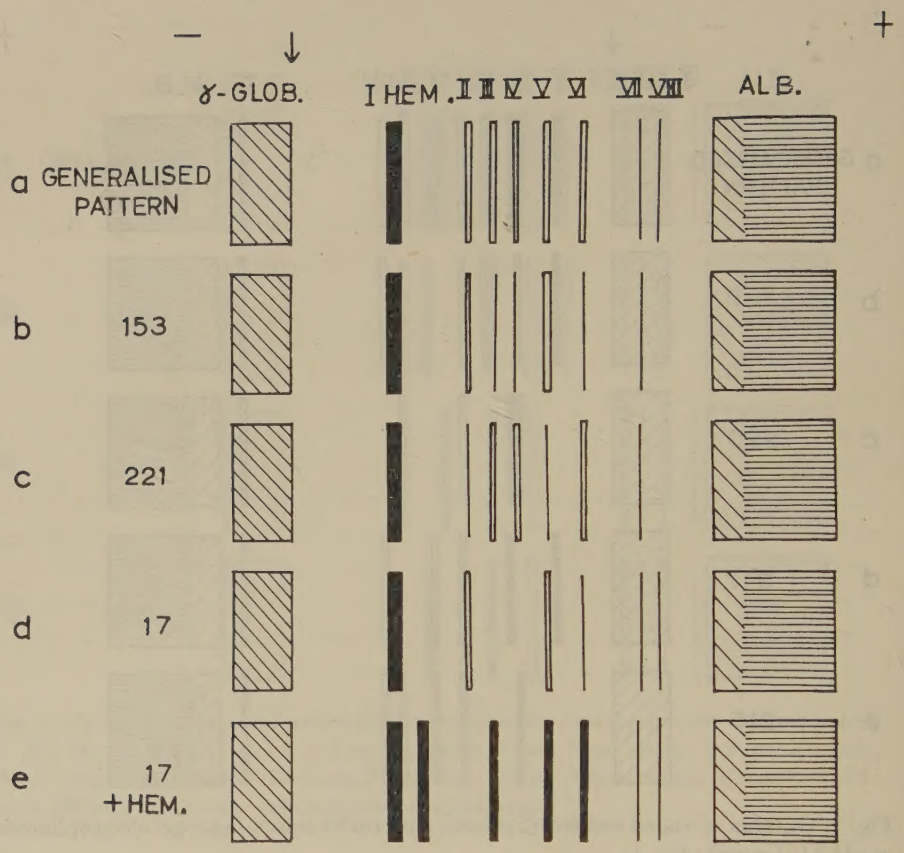

Fig. 3. Outlines of bearded seal serum protein patterns by starch-/agar-gel electrophoresis at pH 9.0. Legend: Fig. 1.

difficult to distinguish between the bands II and III with both techniques. Band I alone was found in «difcoagar» (two specimens), but these sera contained too much hemoglobin to be analysed at pH 9.0.

Individual differencees were also seen for bands IV, V, and VI, but because they were weak, it was difficult to separate the specimens into groups according to these bands.

No clear-cut qualitative difference was found after adding hemoglobins, but the VIII-band stained with benzidine and became stronger when excess hemoglobin was added to the sera, and therefore represents the haptoglobins. Band VII, when it occurred, seemed to become somewhat weaker in these cases.

The weak IX-band was seen in all specimens, but often it was partly screened by the albumins.

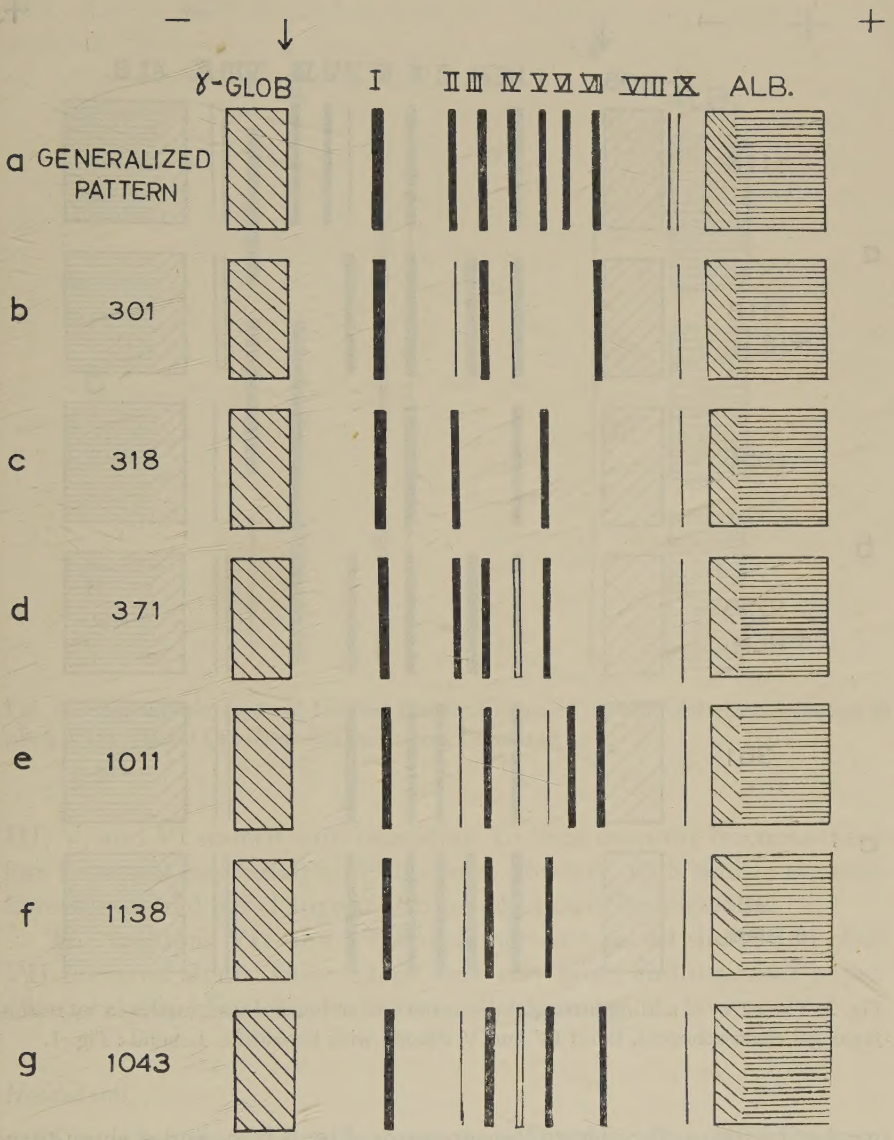

Fig. 4. Outline of hooded seal serum protein patterns by starch-/agar-gel electrophoresis at pH 9.0. Legend : Fig. 1.

Bearded seal

A generalized starch-/agar-gel electrophoresis pattern of the serum proteins of bearded seals is shown in Fig. 3 a. Compared to the other species investigated, the bearded seal serum proteins usually showed

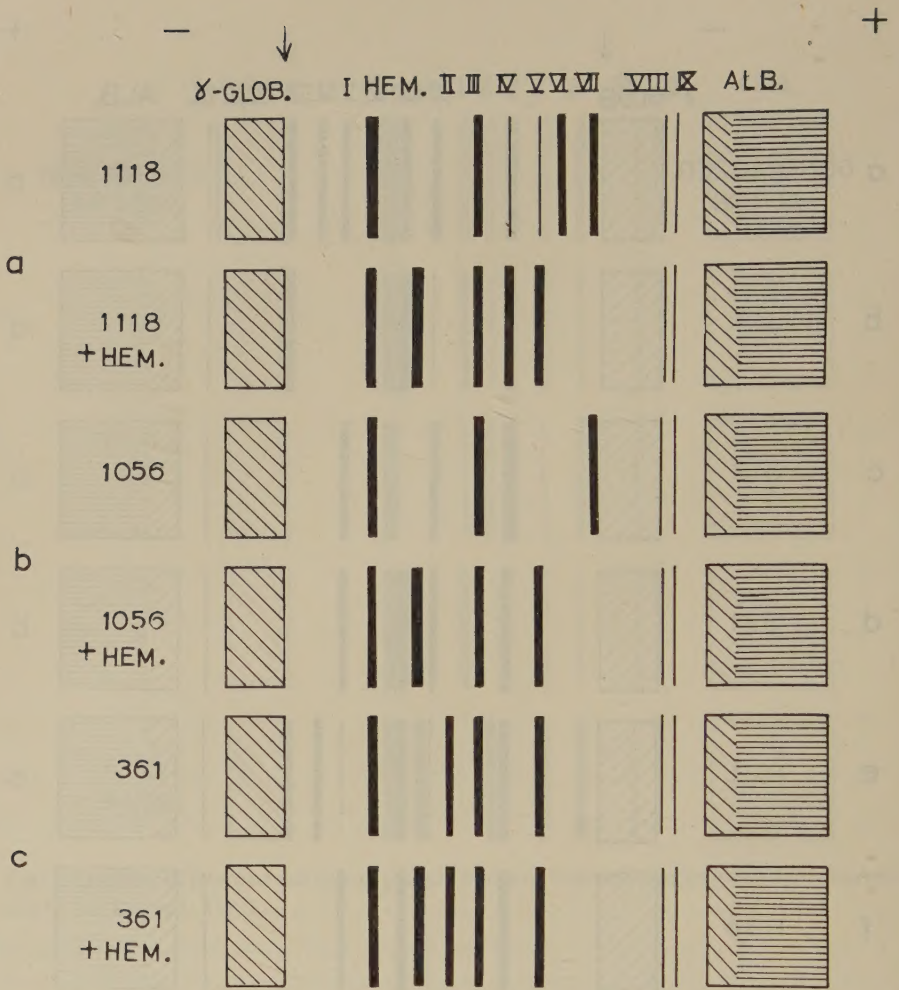

Fig. 5. The effect of adding hemoglobins to the sera of hooded seal, analyzed by starch-/agar-gel electrophoresis. Band IV and V stained with benzidine. Legend : Fig. 1.

weaker bands on the electrophoretograms. Fig. 3 b, c, and d show three individual electrophoretograms from sera free from hemoglobins.

Band I was the the strongest and often had a diffuse appearance which indicates that it may be composed of two proteins.

Band no. II, III, IV, V, and VI appeared with varying strengths, and although it seemed clear that some individual differences excisted in these proteins (Fig. 3 b, c, and d), well-defined results could not be obtained. After addition of hemoglobin to sera, some of the electrophoretograms changed pattern (from d to e in Fig. 3) and the fractions

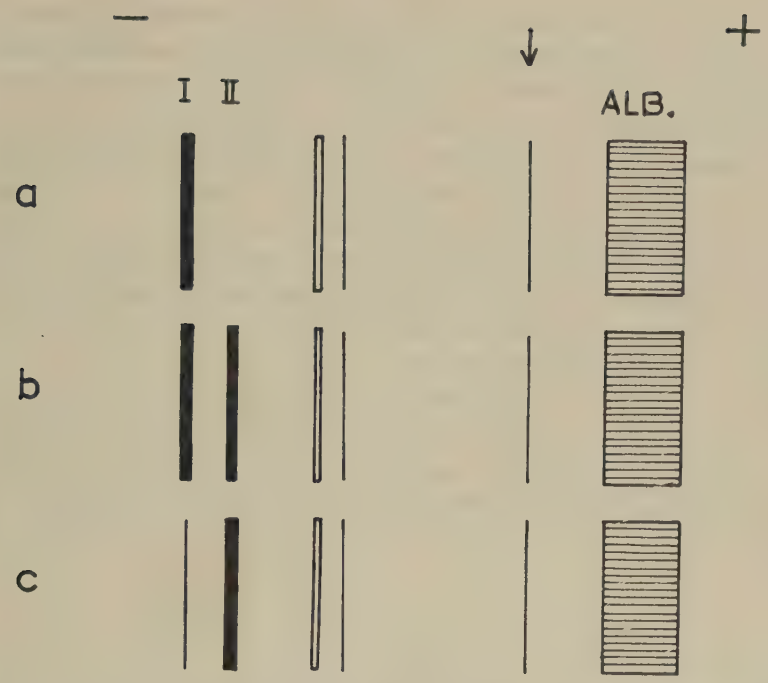

Fig. 6. Haptoglobin types of hooded seal sera revealed by agar-gel electrophoresis at pH 6.3. Only band I stained with benzidine. Legend : Fig. 1.

III, V, and VI stained with benzidine. In these cases the fractions therefore represent haptoglobins. Other sera, however, with similar electrophoretograms did not change at all after addition of hemoglobins.

The fractions VII and VIII were not seen on all slides, and often VII occurred alone. These bands were very faint, and it proved impossible to obtain clear-cut results from all specimens.

Hooded seal

The most complicated patterns were found in electrophoretograms of sera from this species, and several individual differences were discovered. A generalized pattern from electrophoresis in starch-/agar-gel is shown in Fig. 4 a, and six individual patterns of sera which appeared to be free from hemoglobins are shown in Fig. 4 b - g.

Band I, which was demonstrated by autoradiography to represent transferrins, was found in all specimens. In some cases, however, this band was diffuse, indicating a compound of two proteins with similar electrophoretic mobility.

Individual variations were found in band II and III. In some sera both bands appeared with equal strength, in others one of them was lacking. More often, however, the bands appeared with very different strength.

It has been possible to classify the specimens in three groups (phenotypes), II-II, II-III, and III-III respectively, according to the occurrence of these bands. Specimens with only band II or with band II and traces of band III were classified as II-II, when both bands occured with the same or nearly the same strength, the specimens were ascribed to group II-III; and specimens with only band III, or with band III and traces of band II, were classified as III-III.

The bands IV, V, VI, and VII seemed to be closely connected with haptoglobins. The VI and VII-bands, when occurring, disappeared after addition of hemoglobins to the sera before electrophoresis. Band V stained strongly and band IV weakly with benzidine. In Fig. 5 some electrophoretograms of sera before and after addition of hemoglobins are shown.

In sera which apparently were free from hemoglobins, band VI and band VII occurred alone or together. In some sera both bands were lacking. After addition of hemoglobins, these bands disappeared and band V became visible, either alone or together with band IV (Fig. 5 a and b). In specimens without band VI or VII no qualitive changes in the electrophoretograms were detected after addition of hemoglobins, but band V became somewhat stronger (Fig. 5 c).

Using «difcoagar» at pH 6.3, clear individual differences were found in the haptoglobins. The pattern shown in Fig. 6 a was found for sera free from hemoglobins, and all specimens contained the component labelled II. After addition of hemoglobins, however, the specimens could be separated into three groups. In group one no changes occurred, and no bands stained with benzidine. All sera from pups belonged to this group, but the characteristics were also found among older animals. In the second group (Fig. 6 b) a new component (labelled I) occurred while band II persisted although with a somewhat reduced strength. Only band I stained with benzidine. The third group is represented in Fig. 6 c. Here band II disappeared, except for a faint trace, while band I was very strong and stained with benzidine.

It has not been possible to compare these results with the haptoglobin types at pH 9.0.

The faint bands VIII and IX (Figs. 4 and 5) were not found in all specimens. Sometimes only IX occurred, and sometimes both were missing. These bands are very faint, however, and they are located near

the stronger albumins, so the suggested individual differences may be apparent only.

In sera from pups («bluebacks») some of the proteins were found as they occurred in sera from older animals, although often weaker. However, the bands IV - VII were diffuse and very weak in blueback-sera.

DISCUSSION

Except three specimens from harp seals, all analysed hemoglobins have given the same result, and accordingly all the four seal-species seem to be monomorphic in the composition of hemoglobins as far as it can be revealed by this type of electrophoresis. However, «abnormal» hemoglobins like these found in harp seals may also exist in the other three species. Too few specimens have been analysed yet to decide this. The deviating harp seal hemoglobins occur at such low frequencies that even it they are genetically controlled, it is not at all certain that they represent a real case of polymorphism. They might for instance be caused by new mutations which are too unfavourable to be established at high frequencies in the populations.

The transferrin groups of harp seals have been found to be controlled by three autosomal, co-dominant alleles (Nævdal 1966). A similar mode of inheritance is indicated by the I, II, III-fractions of the ringed seal sera, although the specimens from this species are too few to allow statistical tests of the distribution of phenotypes and its accordance with Hardy-Weinberg's law. However, six phenotypes should be expected and five have been found. The expected type III-III is missing, but band III is rare in the available specimens and the expectance of an individual homozygotous for the gene supposed to control it, therefore is very low when the number of specimens is as small as in this case.

Autoradiography has been used only for sera from harp and hooded seals. In both cases the transferrins were found in the fraction or group of fractions with the lowest mobility towards the anode. It is probable that this is also the case for the other two species, and accordingly it is possible that fraction I of the bearded seal sera and fractions I ,II, and III of the ringed seal sera are transferrins, being monomorphic in bearded seals and polymorphic in ringed seals.

Interpretation of the observed individual differences in fractions next to the transferrins in harp, ringed and bearded seals is difficult. For the harp seal very small intraspecific differences have been found in these fractions (band IV), and for the other two species the bands are too weak for classification.

For the hooded seal, however, it is possible to separate the specimens into groups according to the presence or absence of the bands II and III

(although weak bands may cause some difficulties in classification). The frequencies of these proteins or the genes supposed to control them, therefore may be used to investigate the population problem for this species. The provisional results show that the distribution of phenotypes seems to be in good accordance with Hardy-Weinberg's law, provided that these proteins are controlled by two allelomorphic genes.

Blumberg, Allison and Garry (1960) found individual differences in haptoglobins of the fur seal, *Callorhinus ursinus,* and these variations seemed to be under genetic control, although no proof of this could be given. Individual differences in the haptoglobins are very clear in hooded seal, and also indicated in the bearded seal. No interpretation of the bearded seal haptoglobins can be given, because some specimens had three components of hemoglobin/haptoglobin complexes, while others seemed to have none.

The haptoglobin types of the hooded seal, although complicated, are of greater interest because it seems to be possible to use also these characteristics in population studies of the species. There are, however, some problems. The haptoglobin variations at pH 9.0 are best seen when analysed as free haptoglobins (Fig. 5, band VI and VII), but when none of these bands occur, it is often impossible to decide whether they are really lacking or whether traces of hemoglobins bind the haptoglobins as hemoglobin/haptoglobin complexes.

Haptoglobin-bands are very weak in sera from pups, and the hemoglobin-binding capacity seems to be nearly absent in such sera. Therefore mother/pup combinations can not be used to solve the question of heredity for these proteins, and sera from pups can not be used for determination of haptoglobin types. This is unfortunate because pups account for the greater part of the catches and consequently they are the easiest to sample. However, the study of haptoglobins will be continued with sera from adult animals.

Blumberg et al. (1960) described some individual differences in postalbumins of fur seals, and Smithies (1959) has described polymorphism in the postalbumins of man. The variations of the bands VIII-XI for harp seals, VII and VIII for bearded seals, and IX and X for hooded seals may correspond to this, but the bands are very weak.

Some of the individual variations found by starch-/agar-gel electrophoresis at pH 9.0 can also be demonstrated in «difcoagar»-gel at pH 6.3. At this pH the hemoglobins are precipitated in the gel near the application point, and do not disturb the electrophoretograms of serum proteins. During sampling it has proved difficult to avoid all lysis of cells, but with the present methods also sera containing some hemoglobin may be analysed.

The methods are comparatively rapid, permitting analysis of large samples within short periods of time, and this of course is a factor of some importance in population studies.

One disadvantage of the methods is that small amounts of sera are analysed, and some of the individual differences therefore appear only in the presence or absence of very weak fractions, making it impossible to classify all specimens from these characteristics.

Other methods might reveal additional individual differences in hemoglobins as well as in serum proteins, and it would be worth while to try technical modifications. The results obtained with the present methods, however, encourage further investigations, and will be applied in future studies of seal populations.

SUMMARY

Hemoglobins and serum proteins of four species of pinnipeds have been analysed by starch-/agar-gel electrophoresis at pH 9.0, the serum proteins also by agar-gel electrophoresis at pH 6.3.

The original purpose of the work was to discover polymorph characteristics to be used in population studies of the two most valuable species: the harp seal and the hooded seal. During sampling also some specimens from ringed and bearded seals have been collected, and descriptions of the obtained electrophoretograms are given. The greatest attention has been paid to intraspecific variations detectable by these relatively simple electrophoretic methods.

For all species, several individual differences have been found. Particularly the transferrin variations in harp and ringed seals (probable transferrins) and haptoglobin variations in the hooded seal are conspicuous.

Individual differences in other serum proteins have been found for all species, but they are most obvious in the hooded seal. Although no definite proof can yet be offered (except for the transferrins of the harp seal), it is probable that most of the variations are genetically controlled.

The transferrins of harp seals, proteins labelled II and III of hooded seals, and perhaps also the haptoglobins of the hooded seal seem to be suitable characteristics for studies of population problems.

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POLYMORPHISM OF SERUM TRANSFERRIN IN COD

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INTRODUCTION

An ironbinding protein in human serum was found by Holmberg and Laurell (1945). This protein binds ferrous ions in such a way that the iron does not react with $\alpha\alpha'$-dipyridyl, and is now usually called transferrin (Holmberg and Laurell 1947, Giblett, Hickman and Smithies 1959).

Inherited variations in this protein has been revealed by the use of electrophoretic techniques (Tiselius 1937, Smithies 1955) in numerous mammalian species (Schmid 1961, Cooper and Sharman 1964), in chicken (Ogden, Morton, Gilmour and McDermid 1962) and in pigeon (Mueller 1961, Mueller, Smithies and Irwin 1962). Transferrins have also been found in sera of amphibians and reptiles (Dessauer and Fox 1964).

The transferrin polymorphism in species studied has its origin in a genetic system with two or more codominant autosomal alleles, each controlling one or more bands in the electrophoretic pattern. In the macaque, *Macaca mulatta,* ten molecular forms, each controlled by one gene, and 24 phenotypes have been detected (Goodman, Kulkarni, Poulik and Reklys 1965). The transferrin types in Norwegian reindeer are controlled by eight alleles and each allele produces two bands (Brænd 1964).

Fish proteins have been studied by Hamoir (1955), who states that the main characteristics of fish protein are satisfactorily defined, and that a comparison of the highest and lowest classes of vertebrates is possible from the point of view of protein composition. The comparative study of the electrophoretic pattern of plasma or serum shows, however, that the lowest vertebrates apparently do not have common characters *inter se* or with the mammalian ones (Hamoir 1955). Sanders (1964) examined the electrophoretic patterns in sera of three trout species. In two species he found characteristic protein fractions for the particular species. The rainbow trout, however, exhibited either 6 or 7 fractions. The amount of protein differed both inter- and intraspecificly, but he suggested that the amount present varied with age, sex, species, and diet. Khailov

(1962) studied the protein variation in sera from cod, plaice, and haddock in the autumn. He found distinct differences between the species and individual variations of the fractions with time.

A single transferrin band was demostrated in jack (*Caranx sexfasciatus*) by Blumberg (1960). Creyssel, Silberzahn, Ricard and Manuel (1964) found polymorphism of transferrins in carp (*Cyprinus carpio*) by starch gel electrophoresis, and Fine, Drihlon, Amouch and Boffa (1964) detected the presence of several transferrin types in eel (*Anguilla anguilla*) by paper electrophoresis. However, too few carp and eel individuals were studied to decide the heredity with sufficient certainty.

The present paper describes transferrin patterns in cod (*Gadus morhua*) and discusses the genetic basis for the observed differences. The investigation was carried out to identify individual genetic characters for studies of cod populations. Haemoglobin polymorphism has been demostrated in cod, using agar electrophoresis of freshly prepared oxyhaemoglobin (Sick 1961).

MATERIAL AND METHODS

The samples used in the present study are selected from a material collected in 1964 and 1965 for population studies. Unfortunately, part of this material was destroyed before the sera could be analyzed in the laboratory. The samples used are from panmixed populations and from different geographic areas with different frequency of the characters studied from one sample to another. As indicators for panmixing, haemoglobin types (Sick 1961) and otolith types (Rollefsen 1933) were used. Table 1 gives a survey of the material containing six samples, with sera from 682 individuals, listed in a geographical order, starting with the

Table 1. Date, locality, and gear used, of the collected samples.

Date of sampling	Locality	Gear	Number of fishes in sample
18 Dec. 64	Hordaland, coastwards	Trap-net	100
4 Dec. 64	Smøla, coastwards	—»—	100
1 Dec. 64	Helgeland, coastwards	—»—	98
26 Oct. 64	Nordskot, Vestfjorden	—»—	153
1 Nov. 64	Varangerfjorden	Shrimp-trawl	83
19 Nov. 64	Bear Island N73°55'E18°15'	Trawl	148

sample from Hordaland in the south and ending with the sample collected 27 nautical miles SW of Bear Island in the north. Cod of different sex and ega are represented in the material, the majority being 4 to 5 years old.

Blood was obtained by puncture of *bulbus arteriosus* in live cod. After clotting overnight at 2—4°C the samples were centrifuged and the sera pipetted off. The material collected near Bergen was examined fresh, whereas the sera from the northernmost localities were shipped to the laboratory in frozen state (—25°C). Without sufficient capacity for freezing, preservation of the samples from distant fishing grounds is a major problem. In the present investigation cod sera could not be stored in a refrigerator for more than five days without destruction of the transferrin molecules. However, frozen sera gave reliable results even after two or three months, although fresh sera gave a better result.

The electrophoretic technique was a combination of Giri's method (1956 a, b), modified by Sick (1961 and personal communication). The most effective buffer system to enhance individual differences among the protein types was 30.25 g/l Tris (Tris(hydroxymethyl)-aminomethane), 3 g/l EDTA (Ethylenediaminetetraàcetic acid), and 2.3 g/l boric acid (Aaronson and Grønwal 1957). The gel was made by mixing 2% starch (BDH) and 0.8% agar («Ionagar» No. 2, Oxoid) in the buffer and heating for half an hour at 96°C in a waterbath while stirring gently. Microscope slides covered by 2 ml of gel were kept in a refrigerator for a quarter of an hour before use.

Each electrophoretic run lasted for two hours. Voltages of 65 to 70 V between the ends of the filterpapers were applied, giving currents of 6 to 7 mA for each slide. Six slides, each with two specimens, were placed in the apparatus in each run. After fixation the proteins were stained with Amido Black 10B and the gel dried.

In cases where the transferrin patterns have been difficult to determine, usually poorly preserved samples, the sera were tested in a second run. This run often gave a good result, provided that particular care was taken to keep the temperature of the gel low.

In order to identify the transferrin bands the method of Giblett *et al.* (1959) was used with some modifications. Approximately 0.1 ml of ferrous citrate of specific activity (Fe^{59}) 100 μC/ml were added to 0.2 ml selected sera. After half an hour at 2°C the sera were subjected to starch/agar gel electrophoresis.

The numbers of the different patterns observed in each of the six samples have been compared with numbers expected according to the Hardy-Weinberg law of genotype distributions in large random mating populations. The differences between observed and expected distributions were tested by applying a chi-square test.

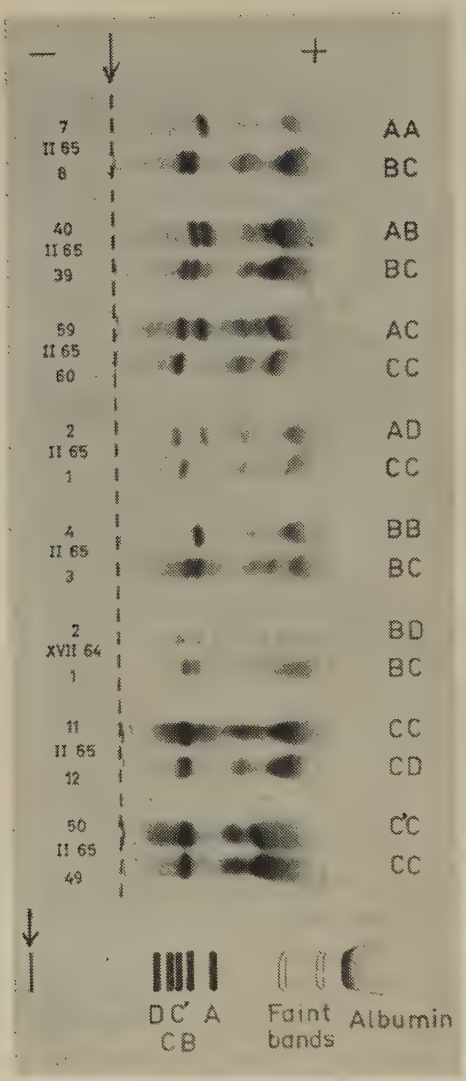

Fig. 1. Starch/agar gel electrophoretograms of cod sera. The arrow indicates the site of sera application, numbers to the left state the number of fish in the sample and the samp'e number, and letters to the right the type of pattern. An enlarged schematic drawing of all components is shown at the bottom of the figure.

RESULTS

Fig. 1 shows ten different patterns made up of eight slides from different runs. An enlarged schematic drawing of all the components is seen at the bottom of Fig. 1. In all cases the proteins move towards the anode. All patterns have a fast moving component (albumin) which frequently reaches the filter paper. Slower moving components of uncertain nature

are seen as one or two faint bands next to the albumin. The components which are studied in this paper are five distinct bands called A, B, C', C, and D; A being the fastest moving component. The bands appear alone or two together, and thus fifteen combinations are possible. Of these eleven have been found. The bands are represented in the serum phenotypes: AA, AB, AC, AD, BB, BC, BD, CC, CD, and C'C. The band D alone has also been found. The mobility for the five components are clearly different, the C' band, however, being only slightly faster than the C band under the electrophoretic conditions used in this investigation. The distance between the stained bands A and B is only slightly longer than the distance between B and C, while this distance is essentially longer than the distance between the bands C and D.

The intensity of the bands vary, and consequently the content of transferrin in cod sera differs from one individual to another. Generally the one band patterns are stronger than the two band patterns. However, visible variations in the amount of protein according to season of the year have not been detected.

The present material contains specimens of different sex and age, and the components A, B, C, and D are represented in every agegroup from 3 to 16, in males and females, and in immature as well as mature cod.

Fig. 2 shows four slides with the three patterns AC, BC and CD compared with autoradiograms of the same slides. The Fe^{59} is bound to the bands described above, and variable quantities of radioactivity appear also in the albumin component except in one case. No radioactivity is bound to the faint bands of uncertain nature.

A hypothesis involving five codominant alleles Tf^A, Tf^B, $Tf^{C'}$, Tf^C, and Tf^D may be adopted to explain the transferrin variation observed. This hypothesis gives the homozygotes Tf^ATf^A, Tf^BTf^B, $Tf^{C'}Tf^{C'}$, Tf^CTf^C, and Tf^DTf^D which are assumed to be responsible for the phenotypes AA, BB, C'C', CC, and DD, giving the hetrozygotes Tf^ATf^B, $Tf^ATf^{C'}$, Tf^ATf^C, Tf^ATf^D, $Tf^BTf^{C'}$, Tf^BTf^C, Tf^BTf^D, $Tf^{C'}Tf^C$, $Tf^{C'}Tf^D$, and Tf^CTf^D for the phenotypes AB, AC', AC, AD, BC', BC, BD, C'C, C'D, and CD. The distributions of the different transferrin patterns from six samples are presented in Table 2 together with the expected distributions according to the Hardy-Weinberg law. The column «Not rep.», contains expected totals of the notrepresented types AC', BC', C'C' and C'D.

The chi-square value for the observed distributions and the expected Hardy-Weinberg distributions in samples from Helgeland, Vestfjorden, and Bear Island, gives a probability between 0.7 and 0.5 when d.f. = 3. According to the test it is therefore no significant deviation between the observed and expected distributions in these samples. The

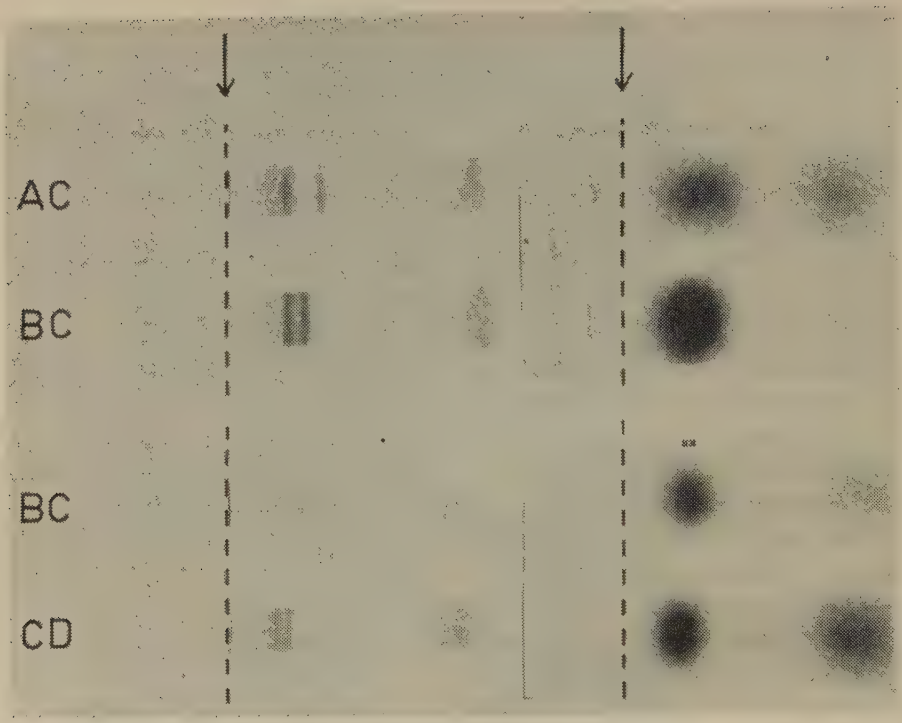

Fig. 2. Starch/agar gel electrophoretograms of transferrin in cod sera (left) localized from autoradiograms (right). Arrows show the site of serum application, and letters indicate the type of pattern.

samples from Hordaland, Smøla and Varangerfjord are omitted because of the low number in the classes of the expected distributions.

The gene Tf^C dominates the material, particulary in samples from southern Norway, and the frequencies of Tf^A (q^A) seem to increase northward, while the frequencies of the Tf^B allele fluctuate between 0.1000 and 0.1735. $Tf^{C'}$ and Tf^D are rare in the areas investigated.

DISCUSSION

The present investigation has demonstrated that cod transferrins are controlled by codominant alleles.

The transferrin patterns consist of one or two strong bands and was not found to vary with the age of the fish. This is in contrast to the variation of the haemoglobin patterns in salmon (Koch, Bergstrøm and Evans 1964), which was found to be ontogenetic. The amount of the cod transferrins may vary with time, though it has not been possible to detect any visible variation by the method used in this study. Khailov (1962) found variations in the amounts of different fractions during

Table 2. Distribution of transferrin phenotypes observed in samples of cod from different areas compared with the expected distribution of genotypes according to the Hardy-Weinberg law.

Sample		Transferrin patterns											Not rep.	Observed gene frequencies				
		AA	AB	AC	AD	BB	BC	BD	CC	CD	DD	C'C		A q	B q	C q	D q	C' q
Hordaland	obs.			3		2	13	3	74	2		3		.015	.100	.845	.025	.015
	exp.	0.02	0.30	2.54	0.08	1.00	16.90	0.50	71.40	4.23	0.06	2.54	0.45					
Smøla	obs.		1	7		1	23	1	63	3		1		.040	.135	.800	.020	.005
	exp.	0.16	1.08	6.40	0.16	1.82	21.60	0.54	64.00	3.20	0.04	0.80	0.20					
Helgeland	obs.		4	14		2	26		48	2		2		.092	.174	.714	.010	.010
	exp.	0.83	3.12	12.86	0.18	2.95	24.29	0.35	50.00	1.43	0.01	1.43	0.56					
Vestfjorden ...	obs.		3	13		4	31		98	4				.052	.137	.797	.013	
	exp.	0.42	2.20	12.76	0.21	2.88	33.49	0.55	97.28	3.19	0.03							
Varangerfj. ...	obs.	2	1	12		2	15		49	2				.102	.121	.765	.012	
	exp.	0.87	2.05	13.01	0.20	1.20	15.31	0.24	48.59	1.53	0.01							
Bear Island	obs.	5	5	29	2	6	27	3	63	7	1			.155	.159	.639	.047	
	exp.	3.57	7.30	29.37	2.18	3.73	30.01	2.22	60.34	8.94	0.33							

the autumn in sera of cod, but he used colourometry to evaluate the different fractions.

The samples were selected to ascertain that the material was from panmixed populations. Samples with distributions of haemoglobin types not in accordance with Hardy-Weinberg law were not used, nor samples with mixed types of otoliths. The material presented was collected during the autumn of 1964, and only one of seven samples has been omitted from the report.

The transferrins are well separated and the patterns of different runs can easily be compared with each other when using this starch/agar gel electrophoresis. The determination of the pattern is not difficult for fresh sera, and the technique is quick and simple, and large samples can be treated.

The determination of the phenotypes has been difficult for poorly preserved samples. If a satisfactory result was not obtained after a second, or perhaps a third run of a serum, the specimen was rejected. Since all sera in a sample were preserved in the same way, they also were either used or discarded together. Methodical selection of the material therefore has been avoided.

It has not been possible to test the rare C' protein autoradiographically. In this report, however, the band C' is interpreted as a very rare ferrous-binding protein because of the appearance of the band: both the position and the strength are comparable to characteristics of the other transferrins. Together with A, B, or D, this band could be difficult to distinguish from AC, BC, or CD. Since C' is rare, however, this is a minor problem, and a higher frequency of C' would primarily give a higher number of the pattern C'C.

Transferrins have earlier been identified in jack, carp, and eel, respectively, by Blumberg (1960), Creyssel *et al.* (1964), and Fine *et al.* (1964). The transferrin patterns studied in this report, however, cannot be compared in detail with those, because different techniques have been used. The position and intensity of a band depend upon the electrophoretic conditions used. The transferrin patterns in carp (Creyssel *et al.* 1964) and in eel (Fine *et al.* 1964) seem to a great extent to be like the patterns in cod, but genetic studies confirming the patterns in carp and eel have apparently not been undertaken. On the other hand, the inheritance of transferrins in cod as a polyallele system without dominance is in strict accordance with results from studies of higher vertebrates (Beckman 1962, Ogden *et al.* 1962).

The variable ironbinding capasity of the albumin component can not be explained by the results in this study. However, the ironbinding cod albumin will be investigated.

The transferrin patterns in cod, which in any case can be separated into four different molecular types named TfA, TfB, TfC and TfD (according to Cohen and Shreffler 1961) represent four codominant alleles Tf^A, Tf^B, Tf^C, and Tf^D. A fifth molecular type called TfC' may represent a fifth allele $Tf^{C'}$.

SUMMARY

Sera from 682 cod from six different localities along the Norwegian coast and in the Barents Sea have been investigated by starch/agar gel electrophoresis. Eleven different patterns composed by five molecular types have been found. By autoradiography it has been demonstrated that four of them are ironbinding proteins. The distributions of the observed transferrin patterns were in agreement with distributions expected according to the Hardy-Weinberg formula, implying that the bands have their origin in five codominant alleles.

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INFLUENCE OF HYDROGRAPHICAL AND METEOROLOGICAL FACTORS ON CATCH AND RECRUITMENT STRENGTH OF THE SPRAT STOCK IN WESTERN NORWAY

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INTRODUCTION

The Norwegian fishery for sprat (*Clupea sprattus* L.) takes place mainly in the Oslofjord and in the fjords of western Norway south of Stad. The season lasts from May to October and by far the greatest part of the catch is taken by purse-seiners and utilized in the canning-industry. During the last ten years the average annual yield has been about 9000 tons.

Various authors have demonstrated relationships between hydrographical factors and recruitment or catch of sprat. Based on observations over five years, Helland-Hansen and Nansen (1909) found that a comparatively small area of coastal water, measured on a vertical section seaward from western Norway, co-occured with small catches of sprat the same year. They also found a correspondence between the dimensions of the area of coastal water in this vertical section and the rainfall in southern Norway and northern Germany the preceding year. Molander (1939, 1943, and 1952) gives several examples of how sprat catches and strength of recruitment depend on hydrographical and meteorological conditions. Along the Swedish west coast easterly and northerly winds caused diminishing catches while southerly and westerly winds were favourable. Years with relatively weak predominant winds had the richest year-classes of sprat. For the sprat stock both along the west coast of Sweden and along the east coast of England, Veley (1951) found that predominant and strong offshore winds were correlated with relatively poor year-classes.

The present investigation is confined to the West-Norwegian part of the sprat stock. Sund (1911) assumed that this sprat mainly originated from spawning grounds in the Skagerak and that the larvae were brought by the coastal current to the fishing area. This view has been supported by Bjerkan (1923 and 1926), even though analysis of vertebrae counts

(Dannevig 1951), egg and larvae investigations (Dannevig 1954, Gundersen 1954), and examination of length composition of the catches (Bjerkan 1950 and 1958) indicated that local spawning in the fjords of western Norway might be of some importance for the recruitment.

The coastal current carries water of low salinity, here called Skagerak water, from the Skagerak westward and northward along the coast of southern Norway. If Sund's assumption is correct, the recruitment to the sprat stock will be largely dependent on the coastal current. The water transport, the direction, and the speed of the current will be among the most important factors influencing the drift of the sprat larvae. If the hydrographical conditions of the coastal current explicitly affect the recruitment to the stock in western Norway, it supports the view that spawning in the fjords may be of minor importance.

In order to compare hydrographical conditions of the coastal current and the recruitment to the sprat stock, an attempt has been made to express numerically : Variations in quantity of Skagerak water from year to year, and variations in strength of recruitment.

Variations in the strength of recruitment could be revealed by determining the abundance of sprat larvae in the coastal current each year. However, the available material is restricted, and an attempt has been made to express recruitment strength by means of sprat catch statistics. It is probable that the annual sprat catches from western Norway can be used assuming that the quantity of a year's catch reflects the recruitment strength the year before.

The calculated hydrographical parameter will to some extent be influenced by meteorological factors such as wind. It is known that northerly winds on the coast of western Norway transport the surface layers of the coastal water away from land (Helland-Hansen and Nansen 1909, Eggvin 1940). In view of this, wind observations have been compared with the calculated average thickness of the Skagerak water.

Bjerkan (1958) states that the northward drift of the sprat larvae is related to the force of the coastal current at the end of the summer, and that this is what causes the fishing districts to shift from year to year. It seems likely that not only the force but also the direction of the coastal current and the wind effects are determining factors in the destination of the larvae. Since few current measurements are available, wind observations have been used to investigate the influence of northerly winds upon the distribution of the catches within the fishing area in western Norway.

Table 1. The mean depths of the 34‰ isohaline in the summer months at Utsira and the sprat catches in western Norway. Mean depth in metres, catch in 1000 "skjepper".

Year	Mean depth d	Catch c
1942	41.6	—
43	41.1	488
44	31.1	346
45	42.0	319
46	45.9	592
47	29.9	327
48	38.1	213
49	30.7	393
50	38.1	208
51	36.4	336
52	35.9	213
53	46.8	392
54	40.7	390
55	31.2	166
56	46.8	104
57	37.7	438
58	50.7	144
59	35.9	441
60	32.9	379
61	54.1	240
62	38.5	549
63	39.8	804
64	39.8	539
65	—	308
	$\bar{d} = 39.38$	$\bar{c} = 362.1$
	$s_d = 6.385$	$s_c = 163.46$

MATERIAL

Since 1942 the Institute of Marine Research has collected data, usually every fortnight, at a fixed oceanographic station five nautical miles southwest of Utsira Island (59°15' N, 4°47' E). Dr. Jens Eggvin has kindly made salinity isopleth diagrams from this station available. The area on the Utsira isopleth diagrams enclosed by the surfaceline and the 34‰ isohaline depth between the dates 1 May and 30 September

was measured with a planimeter. From the values obtained the mean depth of the $34^0/_{00}$ isohaline each year was calculated (Table 1). This expresses the average thickness of the Skagerak water in the summer months.

In Table 1 is given the sprat catches in western Norway in "skjepper", (one "skjeppe" is equivalent to 17 kg and is the Norwegian standard unit of sprat measurement).

The wind data used have been collected at the meteorological station on Utsira Island (59°18' N, 4°53' E) and reported in Jahrbuch des Norwegischen Meteorologischen Instituts 1942—1944 (1944—1945) and Norsk Meteorologisk Årbok 1945—1964 (1946—1965). The wind observations of each year have been transformed into a wind-vector. The wind-vector comprises the products based on monthly mean wind forces and the number of cases with wind from north, summed over the months May, June, July, August, and September. The wind forces are given according to the Beaufort scale. The wind directions for the years up to and including 1948 are given in the eight general directions, later in the scale 01—36 reduced to the nearest direction divisible by three. This means that the wind-vectors for the period 1942—1948 cover a sector of 45° around north and for 1949—1964 a sector of 30°.

RESULTS

In Fig. 1 the mean depth of the $34^0/_{00}$ isohaline one year is compared with the the sprat catch in western Norway the following year for the period 1942—1965. Years having relatively great mean depths are as a rule followed by years giving large catches of sprat.

The relationship demonstrated in Fig. 1 has been correlated statistically for the 23 years with interdependent sets of data, *c* and *d* (Table 1). Calculation of the correlation coefficient gave $r_{c,d} = 0.4713$, ($P \approx 0.02$).

The result indicates that there is a certain linear correlation between the two factors, which means that good catches seem to be conditioned by a thick layer of Skagerak water the previous year. But the value of the coefficient shows clearly that the interdependence is restricted.

From the residual variance $s_c^2 = s_c^2 (1—r_{c,d}^2) = s_c^2 \cdot 0.77$ it may be seen that a great part of the variance of the catch is caused by other factors than those expressed in the mean depth of the $34^0/_{00}$ isohaline. Only about 22 % of the variance may be explained as due to changes in the thickness of the Skagerak water near Utsira.

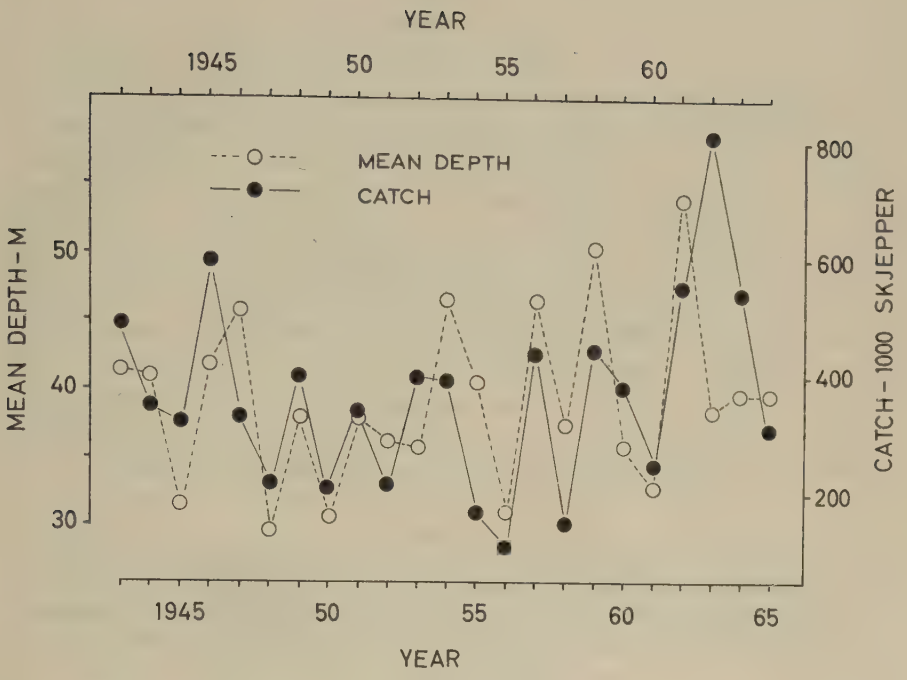

Fig. 1. The mean depth of the $34^0/_{00}$ isohaline at Utsira in the summer months compared with sprat catch in western Norway the subsequent year.

In Fig. 2 the thickness of the Skagerak water is compared with the calculated wind-vectors of northerly wind the same year. The figure indicates a restricted relationship between the mean depth of the $34\,^0/_{00}$ isohaline and the wind-vector, showing that small wind-vectors give great thickness and conversely. Consequently, in view of Fig. 1, northerly wind may be a primary factor in determining the recruitment strength by influencing directly the transport of sprat larvae. However, a comparison of wind-vectors and catch gives a lower correlation than that obtained between the thickness of Skagerak water and catch.

In Fig. 3 the wind-vectors are compared with the sprat catch in Rogaland which is the southernmost county within the fishing area in western Norway. The annual catch in Rogaland is expressed as a percentage of the total catch in western Norway and the wind-vectors of one year are compared with the catch percentage the subsequent year.

The comparison indicates that when weak northerly winds predominate during summer a greater part of the total sprat catch will be taken in Rogaland the following year.

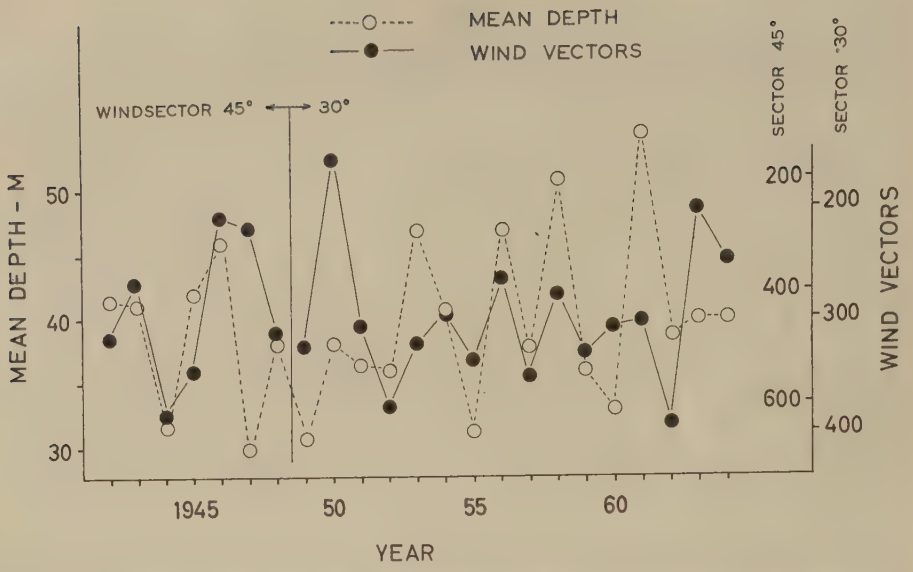

Fig. 2. The mean depth of the $34^0/_{00}$ isohaline at Utsira in the summer months compared with wind-vectors of northerly winds. The wind-vectors for the period 1942—1948 cover a sector of 45° around north and for the period 1949—1964 a sector of 30°.

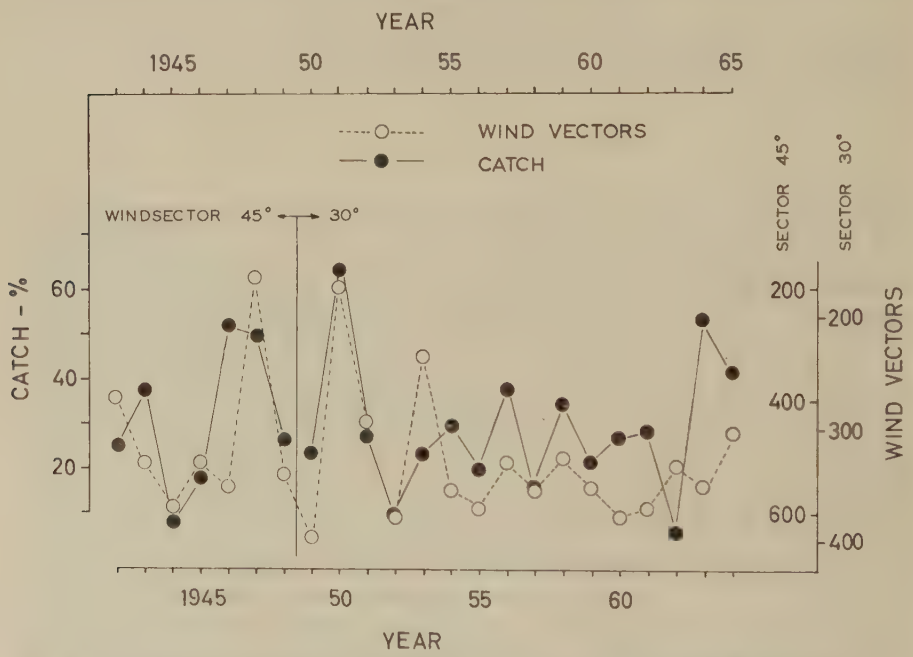

Fig. 3. Wind-vectors of northerly winds compared with sprat catch in Rogaland county the subsequent year as percentage of total sprat catch in western Norway. The wind-vectors for the period 1942—1948 cover a sector of 45° around north and for the period 1949—1964 a sector of 30°.

DISCUSSION

The fixed oceanographic station near Utsira has a position well suited for the investigation. The observations are taken within the coastal current between the two principal sprat fishing districts in western Norway, the Hardangerfjord area and the Ryfylkefjords; the depth here is more than 200 m. The observations from the station do not give information about the direction and speed of the coastal current and tell little about the horizontal extent of the Skagerak water. Variations in the thickness of Skagerak water, however, are clearly demonstrated from the observations.

Salinities below $34^0/_{00}$ are characteristic for Skagerak water (Laevastu 1963). This is also the limit for the *coastal water* as used by Helland-Hansen and Nansen (1909), and they point out that the variations in vertical and horizontal extent are best demonstrated by choosing this limiting value.

The period 1 May - 30 September covers the time when sprat larvae are likely to appear along the coast of western Norway, assuming recruitment from spawning areas in the Skagerak and the Kattegat with spawning optimum in May and June (Höglund 1938, Lindquist 1961).

Outside western Norway very few sprat larvae have been found (Ljøen 1962). Later investigations (present author, unpublished) gave evidence that sprat larvae of more than 20 mm length avoid the plankton nets used. Further east in the coastal current the larvae are smaller and data from plankton net surveys are more reliable. Dannevig (1956) investigated the outer Norwegian coastal waters in the Skagerak south of Ferder and between Larvik and Kristiansand taking vertical hauls from 50 m to the surface with a one metre plankton net. His data give the following averages :

1950	13.4	sprat larvae per haul
1951	2.1	— » —
1952	4.6	— » —
1953	46.7	— » —
1954	42.5	— » —

There seems to be no relationship between the average number of larvae per haul one year and the catch of sprat in western Norway the following year. For example, the number of larvae in 1953 was ten times that in 1952, but the corresponding catches of sprat in 1954 and 1953 were almost similar.

The assumption that the sprat catch directly reflects recruitment strength is based on particular facts regarding the sprat fishery. Between 1920 and 1930 more than 95 % of the sprat catch consisted of one year old sprat, after 1930 two year old sprat have been found more frequently

(Bjerkan 1950). Yet it may be assumed that the fishery in the years 1943 to 1965, which are covered by this investigation, has mostly been concentrated on single year-classes. Samples taken from sprat catches during the last decade show length compositions which support this view (Gundersen, personal communication).

Table 1 illustrates considerable variations in annual yield of the West-Norwegian sprat fishery. Several factors may influence the catches.

The abundance is probably directly dependant on the strength of recruitment the year before, because the fishery is mainly restricted to only one year-class. This gives the pronounced annual variations in the sprat fishery.

The availability does not change much from year to year, and this factor has a small influence on the catch. The sprat fishery takes place in inshore waters nearly independent of wind and weather conditions.

The effort in the fishery cannot be expressed by exact data as participation, fishing period, equipment, etc. are not specified in available statistics. Sild- og Brislingsalslaget, Bergen, which is the sales organization of the sprat fishermen, claims that about 300 purse-seiners take part in the fishery every year. The number has varied little, and was the same even during World War II. During the years covered by this investigation, the efficiency of the sprat fishery has increased due to better boats, synthetic fibre seines, echo sounders, sonar and fishery by artificial light. The number of land-seines has decreased and participation is lower to-day than 20 years ago. However, the average annual yield for the whole country has changed very little. For the decades after 1911 it has been:

1911—1920	582 000	skjepper
1921—1930	604 100	»
1931—1940	483 000	»
1941—1950	483 100	»
1951—1960	492 900	»

This indicates that the increased effiency has not affected the catch.

An evaluation of the factors influencing the catch indicates that abundance is the decisive factor, and that the abundance in turn is mainly determined by the strength of recruitment the year preceding the fishing season.

Weak northerly winds during summer seem to be favourable to the sprat catches the following year in the southernmost district within the fishing area (Fig. 3). According to Eggvin (1940), coastal water masses transported out from land by prevailing winds and pressed northward are later again found near land. This may explain the distribution of the sprat fry and thereby the catch, taking into consideration that the fry is limited to these upper water layers.

Based on the various comparisons the following explanations seem plausible: Years having a weak water transport and much northerly wind in the summer will show a restricted thickness of Skagerak water on the isopleth diagrams. The upper water layers are shifted out from land and the $34^0/_{00}$ isohaline is "lifted". In years when the coastal current carries much water and there is less northerly wind, the thickness of the Skagerak water will be relatively great. Large water transport will increase the chances for transport of sprat larvae from the spawning area in the Skagerak and the Kattegat to western Norway. If the surface layers where the larvae stay are transported seawards, it is possible that under such conditions larvae will not be available to maintain the stock in western Norway. Conversely, a powerful coastal current and weak northerly wind in the summer should favour the influx of larvae and thus increase the sprat catch the following year.

HELLAND-HANSEN and NANSEN (1909) concluded that a great volume of coastal water gives a good catch of sprat the *same* year (see p. 63). This does not agree with the present investigation. A correlation analysis between the thickness of Skagerak water and the catch from the same year indicates no relationship.

CONCLUSIONS

The results from the various comparisons between hydrographical data and catch and catch distribution indicate that the yield of the sprat fishery in a particular year is influenced by current and wind conditions during the preceding year. These relationships suggest that such abiotic factors play a relatively significant part in determining the recruitment strength compared to presumptive important biotic factors, such as success in spawning, food, predators etc.

The indicated relationships support the view that the sprat in western Norway originate from spawning grounds in the Skagerak and the Kattegat.

The correlations are too weak to form the basis for any forecast of the sprat catch.

SUMMARY

The dependence of recruitment strength on hydrographical conditions has been studied, *i. e.*, possible relationship between the transport of sprat larvae from the Skagerak to the stock in western Norway and the thickness

of Skagerak water in the coastal current. The material consists of data from 23 years of hydrographical observations and catch statistics. A correlation analysis gave the correlation coefficient $r=0.4713$ ($P\approx 0.02$). which means that good catches seem to be conditioned by a great thickness of Skagerak water the previous year.

The validity of the material is discussed, especially the use of catch statistics as numerical expression of recruitment strength.

An analysis of the influence of prevailing winds on the hydrographical conditions and on the catch distribution has been carried out. Weak northerly winds during summer seem to give the southernmost county in western Norway a greater proportion of the total sprat catch the following year.

The results of this investigation indicate that the majority of the sprat in western Norway originate from spawning in the Skagerak and the Kattegat.

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